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TI The uptake pathway of DNA and lipids in cationic liposome-mediated gene transfer

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AB The uptake pathway of DNA during the cationic liposome-induced transfection procedure is investigated by fluorescence and confocal microscopy. The cationic lipid, dioleoyl trimethyl-ammonium propane (DOTAP), is mixed with either dioleoyl-phosphatidylethanolamine (DOPE) or dioleoyl-phosphatidylcholine (DOPC), at 1:1 ratio, to form cationic liposomes. These cationic liposomes form DNA: cationic liposome complexes with the plasmid pSV-ss-galactosidase, which is used to transfect CHO cells. Freeze fracture electron microscopy shows that the morphol. of DNA:cationic liposome complexes is an aggregate of DNA and cationic liposomes, with most of the visible, bilayer-ensheathed DNA strands attached to the outside of cationic liposomes. Granule formation from fluorescently labeled complexes was obsd. either in transfection medium, as in the case of complexes contg. DOPE, or on the cell surfaces, as in the case of those contg. DOPC. The transfection efficiency increases with the d. of granules found on cell surfaces. Serum inhibits granule formation also inhibits transfection. Confocal microscopy of cells treated with doubly labeled DNA and lipid complexes reveals that internalized granules contain both DNA and lipid, but those on cell surfaces are mostly DNA aggregates, presumably remaining after cationic liposomes in the complexes fuse with plasma membranes. Labeled lipids begin to transfer to cell membranes immediately after the complexes are added to cells, but the transfer is significant only after an hour of treatment. Inhibition of endocytosis by low temp. or by 20 .mu.g/mL of **cytochalasin**-B reduces granule formation on cell surfaces, and suppresses **transfection**. The results indicate that, for CHO cells, the major uptake pathway of DNA in the cationic liposome-induced transfection process is endocytosis. In the case of PC-contg. cationic liposome, the formation of DNA:cationic liposome granules on cell surfaces is a cytoskeletal directed membrane event akin to the capping phenomenon, and the formation of granules may trigger endocytosis and consequent transfection. This process is more effective than the spontaneous granulation of PE-contg. cationic liposomes in terms of effecting transfection. Thus granule-related endocytosis is a more important factor than membrane fusion in cationic liposome-induced gene transfer into CHO cells.